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Pathophysiology of *Campylobacter* Enteritis

RICHARD I. WALKER,[1] M. BLAKE CALDWELL,[2]* EILEEN C. LEE,[2] PATRICIA GUERRY,[2] TREVOR J. TRUST,[3] AND GUILLERMO M. RUIZ-PALACIOS[4]

Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814-5145[1]; Naval Medical Research Institute, Bethesda, Maryland 20814-5055[2]; Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2[3]; and Department of Infectious Diseases, Instituto Nacional de la Nutricion, Salvador Lubiran, Calle Vasco de Quiroga 15, Delegacion Tlalpan, 14000, Mexico D.F.[4]

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INTRODUCTION

Campylobacter jejuni has exploded from obscurity to be recognized as a major human enteric pathogen. This recognition has triggered numerous bacteriological investigations, but the many mysteries and surprises associated with the organism will challenge the creative efforts of scientists for years to come.

Vibrio (now *Campylobacter*) *fetus* was first isolated in 1909; it was associated with abortions in sheep and cattle. It was not until 1947, when it was cultured from human blood (182), that its potential significance in human disease was appreciated. Over the next decade it was recognized as an opportunistic pathogen of debilitated patients (21, 61). The possibility that *V. fetus* could be also associated with enteric disease was first raised by Elizabeth King, who in 1957 observed that *V. fetus* isolates could be divided into two groups on the basis of thermophilic characteristics. The bloodstream isolates that grew best at 42°C were from patients with preceding diarrheal illness (79, 80), but to accumulate data that associated the thermophilic vibrios with diarrheal disease, the development of a selective medium for the isolation of these fastidious organisms from stool samples was necessary (41). Since then, many epidemiological studies, particularly those of Butzler and Skirrow (29), Blaser et al. (9), and Skirrow (158) have confirmed the importance of King's "related vibrios" as one of the major bacterial enteric pathogens worldwide. This clinical importance, documented in many reviews (20, 35, 43, 99, 139, 163), led to a number of attempts to unravel the pathogenic features of the organism and its disease. Although much controversy remains, considerable progress has been made in recent years toward an understanding of the pathogenesis of campylobacter enteritis. This review presents an overview of that progress.

THE NATURE OF THE PATHOGEN

Although initially classified in the family *Vibrionaceae* because of its morphology, *V. fetus* is unrelated to vibrios on the basis of its different nucleotide base composition (mol% G+C), as well as its inability to use sugars either oxidatively or fermentatively (1, 89, 130, 153). In 1963 a new genus, *Campylobacter*, in the family *Spirillaceae*, was created to include *V. fetus* and related organisms (153, 181). Although the genus contains a variety of species recognized as animal and human pathogens, the most commonly considered as human enteric pathogens are *C. fetus*, *C. coli*, *C. laridis* (170), and *C. jejuni*. DNA hybridization studies by numerous workers have shown that these four are distinct species, sharing less than 35% nucleotide similarity under stringent hybridization conditions (6, 64, 88, 129, 179). Of these four species, only *C. jejuni* has been studied in detail in terms of pathophysiology. For this reason and because it is the species most frequently isolated from diseased individuals in most geographic areas, the rest of this review will center on this organism.

* Corresponding author.

C. jejuni is characterized by a rapidly darting motility due to one or more amphitrichous flagella. Pleomorphism is common among these gram-negative cells. Log-phase cultures contain curved or spiral rods that vary in length from 1.5 to 3.5 μm and in width from 0.2 to 0.4 μm, but as cultures age, round forms may predominate.

C. jejuni grows best in microaerobic environments (i.e., 5 to 10% oxygen, 3 to 10% carbon dioxide) (22) at 42°C. It will grow on a variety of basal media including brucella and Mueller-Hinton agars (127). However, optimal isolation from routine specimens requires the addition of selective (57) and nutritional supplements. The most commonly used nutritional supplements include whole and lysed animal blood along with ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP) supplement (53). More recently, a blood-free selective medium has been described by Bolton and Coates (23). Tenover et al. (175), using a modification of a chemically defined medium originally developed for gonococci, showed that 58% of examined *C. jejuni* isolates are auxotrophic for at least one amino acid and demonstrated the usefulness of an auxotyping scheme in epidemiological studies of *C. jejuni*.

ESTABLISHMENT OF INFECTION

Infective Dose

Infection with *Campylobacter* spp. occurs by ingestion, most often of contaminated liquid or solid food (9, 11, 54, 146, 154). *C. jejuni* is rapidly killed by hydrochloric acid at pH 2.3, indicating that gastric acid is an effective barrier against infection (15). It can survive for 2 to 5 weeks in bovine milk or water kept at 4°C, indicating that the vehicle may be important in determining the infective dose. The most common sources include unpasteurized milk, raw or partially cooked poultry, and contaminated water. Not all exposed persons develop signs of gastroenteritis. For example, a contaminated water supply in Vermont caused 20% of the population of a city to develop the disease (183), but the percentage of the population exposed may have been higher. In milkborne outbreaks elsewhere, one-quarter of the culture-positive population was found to be asymptomatic (140).

Palmer (132) reported a waterborne outbreak of *Campylobacter* gastroenteritis at a boys' school in England; the outbreak was associated with avian fecal contamination of a water-holding tank. This epidemiologic study led to the estimation that as few as 500 organisms initiated the illness (Andrew Pearson, personal communication). Later studies in volunteers (Robert Black, personal communication) showed a variable range in the infecting dose, and many volunteers developed no illness. These data are consistent with a report of illness resulting from 10^6 organisms in a glass of milk (166) and with results of another experiment in which disease was caused in a single volunteer by 500 cells (145). It appears that considerable variation exists either in individual susceptibility to the organism or in the relative virulence of strains, or both.

Motility and Chemotaxis

Once past the gastric barrier, the pathogen must reach and colonize the mucosal surface if the disease processes are to be initiated. The importance of flagella in this process has been demonstrated for *Vibrio cholerae*. Yancey et al. (190) found that nonmotile cholera mutants placed in ligated ileal loops of adult rabbits required at least a 100-fold-higher dose than their respective motile parent strains to produce comparable accumulation of fluid. Other results suggest that flagella can facilitate the colonization by *V. cholerae* through enhancement of attachment (4, 60).

Motility directed by chemotactic stimuli can increase the effectiveness of mucosal colonization. Freter et al. (51, 52) showed that nonmotile or nonchemotactic mutants of *V. cholerae* penetrated the mucus overlying the epithelial surface at the same rate as did inert particles. However, chemotactic motile organisms reached the deep intervillous spaces in ligated rabbit ileal loops at a rate 10-fold higher than that of inert particles. Similarly, in germfree mice, chemotactic strains of *V. cholerae* outgrew nonchemotactic organisms (52).

Hugdahl and Doyle (65) studied the chemotactic behavior of *C. jejuni* and found that positive chemotactic responses were directed toward only L-fucose (of 20 carbohydrates tested) and L-aspartate, L-cysteine, L-glutamate, and L-serine (of 15 amino acids tested). The organism was also attracted to pyruvate, succinate, fumarate, citrate, malate, and α-ketoglutarate. Mouse intestinal mucus and hog gastric mucin were also chemoattractants for *C. jejuni*; this effect may be associated with L-fucose and L-serine components. *C. jejuni* seems to have an affinity for mucus (91), since mice challenged via the orogastric route showed heavy colonization of mucus-rich cecal crypts.

Adherence

Although *C. jejuni* lacks fimbriae, it may possess other adhesins. A variety of studies with cell lines, particularly HeLa and INT 407 cells, have demonstrated in vitro adherence by the organism. In addition to cell lines, *C. jejuni* also adhere to porcine brush border preparations (120).

Numerous substances and treatments can interfere with the adherence of *C. jejuni* to epithelial cells. L-Fucose and asparagus pea lectin (which recognizes L-fucose determinants on cells) inhibit binding to INT 407 cells (36). Similar results were obtained by McBride and Newell (104), who also reported the partial inhibition of binding with other carbohydrates such as glucose, galactose, mannose *N*-acetylglucosamine, *N*-acetylgalactosamine, and the nonsugar carbohydrate sorbitol. McSweegan and Walker (111) reduced the adherence of *C. jejuni* to INT 407 cells by at least 50% through treatments with D-mannose, D-(+)-fucose, 2.5% glutaraldehyde, and proteolytic enzymes.

The flagellum may contain adhesins for epithelial cells. An aflagellated variant of *C. jejuni* was reported to adhere poorly to INT 407 cells (104), which suggests the presence of adhesins on flagella such as are seen in *V. cholerae* cells (4). Although flagellated organisms attach better to cell monolayers, aflagellated organisms attach better than the parental flagellated type to target cells in suspension, suggesting the possibility of multiple adhesins (104). The partial inhibition of adhesion by various agents also suggests a variety of receptors for *C. jejuni* (111). Heat (100°C for 30 min) did not modify adhesion by *C. jejuni*, suggesting the involvement of a determinant other than the heat-labile flagellar protein or the uncovering of latent adhesins.

Other surface structures of *C. jejuni* that could be important in adhesion to the epithelium are outer membrane proteins (OMPs), lipopolysaccharide (LPS), and glycocalyx material. McSweegan and Walker (111) were able to show that tritiated OMP and LPS extracted from *C. jejuni* adhere to INT 407 cells. If an adhesin is determined to be LPS, it would mean that *C. jejuni* possess at least one adhesin

mechanism similar to those described for *Shigella flexneri* (69) and fecal *Escherichia coli* isolates (37).

Intestinal mucus gel is a major barrier to penetration by enteric organisms, but locomotion aided by the spiral shape of *Campylobacter* spp. may facilitate penetration through this viscous matrix. Many bacteria in the intestine associate with the mucus layer (38), and Lee et al. (91) found this material to be a major site for colonization by *C. jejuni*. Freter et al. showed that cholera vibrios added to intestinal slices *in vitro* associated mostly with the mucus gel rather than with the mucosal epithelium (51). McSweegan and Walker (111) found that rabbit mucus gel films prepared as described by Laux et al. (87) effectively bound *C. jejuni*. However, some strains appear to bind better to cells than to mucus, and vice versa. Variation among strains in cell and mucus adherence assays suggests that different adhesins for the two substrates may be involved.

DISEASE IN MAN

Once established, infection can be manifest in several different forms. Disease due to *C. jejuni* infection is most commonly gastrointestinal in nature. However, extra intestinal infections including meningitis (177), cholecystitis (39), and urinary tract infection (40) have been reported. Complications that have been associated with *C. jejuni* enteritis include Reiter's syndrome (70), reactive arthritis (45), and Guillain-Barre syndrome (76, 143).

The incubation period is variable (1 to 7 days), and the diarrhea is usually self limited, lasting 2 to 7 days, but persistent and relapsing symptoms are well reported (20). Excretion of the organism varies from 2 weeks to 3 months in immunocompetent hosts not treated with antibiotics (18). Whether the patient with simple diarrhea should receive antibiotics remains controversial (3, 9, 131). Clinically, *C. jejuni* infection can present as watery diarrhea, a dysentery-like syndrome mimicking inflammatory bowel disease, or, rarely, as an extraintestinal infection. The mechanisms by which *C. jejuni* cause diarrhea have been postulated from studies of the clinical syndromes.

In a recent review, Levine et al. (93) catagorized enteric pathogens into five groups on the basis of the mechanisms by which they produce disease. *Campylobacter* spp. have clinical characteristics that resemble pathogens in three of these groups. One type of mechanism involves toxin-induced disease, which is classically represented by *V. cholerae*. These organisms adhere to the mucosa in the proximal small intestine and elaborate a toxin, resulting in a secretory diarrhea. Toxin production is a proposed mechanism in patients with acute watery diarrhea. The recent demonstration of enterotoxin production by *C. jejuni* (discussed below) has supported this mechanism in the pathogenesis of *C. jejuni*. However, Mathan et al. (102) recently compared toxin production in isolates from children with acute diarrhea and symptom-free carriers in Southern India. They found no difference between the two groups in the percentage of strains that were enterotoxigenic.

Another type of disease, typified by *Shigella* spp., involves penetration and proliferation within the intestinal epithelium. Cell damage and death occur, but the lesion remains superficial, and mesenteric adenitis and bacteremia seldom occur. The terminal ileum and colon are primarily involved, and clinically the stools contain blood and inflammatory cells. In *Campylobacter* enteritis, clinical evidence exists for intestinal epithelial invasion in cases with bloody diarrhea and inflammatory cells in the stool. The infection appears to most commonly involve the terminal ileum and colon. Rectal biopsy was abnormal in eight consecutive patients with *C. jejuni* enteritis studied by Price et al. (142). The lesions consisted of inflammatory infiltrates in the lamina propria and crypt abscesses similar to those seen in infection with *Shigella* and *Salmonella* spp. (17). Similar biopsy results have been reported by others (44, 86). However, the frequency of infection in the proximal small bowel is unknown. King reported hemorrhagic necrosis of the ileum and jejunum found at autopsy of a patient with campylobacter enteritis (80), and Ward et al. recovered *C. jejuni* from a jejunal aspirate (184). To our knowledge, no one has prospectively investigated small-bowel biopsies in patients with campylobacter enteritis.

A third mechanism, termed translocation, is characterized by *Salmonella* and *Yersinia* spp. In translocation the organisms penetrate the intestinal mucosa, resulting in minimal damage, and proliferate in the lamina propria and mesenteric lymph nodes. The involvement of *C. jejuni* with the mesenteric lymph nodes is well described clinically (29, 158). Pearson et al. (133) reported that six of 251 children who underwent surgery for acute appendicitis at Southampton General Hospital, Southampton, England, had *C. jejuni* isolated from appendix tissue, rectal swabs, or peritoneal fluid. Three of these had abnormal appendices at the time of surgery, and three had mesenteric adenitis. Recently, Yousef et al. (192) showed that in gnotobiotic mice, *C. jejuni* translocates to the mesenteric lymph nodes. Evidence indicates that translocation is a mechanism by which *C. jejuni* causes disease and that the frank dysentery and profuse watery diarrhea may represent two ends of the clinical spectrum. Alternatively, the variation in symptoms may be the result of different mechanisms predominating for different strains, such as those seen for various types of *E. coli* (e.g., enteropathogenic, enterotoxigenic, and enteroinvasive) enteritis.

It remains to be determined whether the variability in the disease presentation is due to inherent differences among strains of *C. jejuni* or to host responses. Recently Klipstein et al. (84), using fresh isolates, were able to show differences among strains isolated from patients with asymptomatic illness, watery diarrhea, or dysentery. Six isolates from patients with bloody diarrhea elaborated cytotoxin as measured in Vero and HeLa cell assays, but broth filtrates from these organisms did not cause fluid accumulation in ligated rat ileal loops. Six strains isolated from patients with watery diarrhea produced an enterotoxin and caused fluid secretion in ligated rat ileal loops, but only one of these isolates elaborated a cytotoxin. Eight strains isolated from asymptomatic carriers were negative in all assays. Further work in this area may help to determine the specific virulence factors of the organism.

The prevalence and significance of bacteremia associated with *C. jejuni* enteritis have been controversial. Bacteremia in otherwise healthy patients has been reported (43, 98). However, when systematically sought, it has not been demonstrated. Human volunteers had serial blood cultures drawn after being experimentally fed with *C. jejuni*, and no bacteremia was detected (Robert Black, personal communication). Recent evidence indicates that bacteremia may be due to decreased sensitivity of specific organisms to serum rather than to differences in invasive properties (19, 74). *C. jejuni* and *C. coli* are generally serum sensitive in the absence of specific antibody, but isolates from blood are less serum sensitive than those from stool. LPS composition may be an important determinant of serum susceptibility among *Campylobacter* spp. (137).

Another possible factor in *Campylobacter* virulence was recently described by Kiehlbauch et al. (78), who demonstrated that *C. jejuni* survived intracellularly in peripheral blood monocytes in vitro for up to 6 to 7 days. The observation that these organisms survived longer than control organisms grown in the absence of phagocytes led these authors (78) to postulate that phagocytosis may actually facilitate the survival of *C. jejuni*. Survival within macrophages is thought to be one of the primary virulence determinants in infections with *Salmonella typhi*. After penetration of the intestinal epithelium, the organisms are ingested but survive within the macrophage while being dispersed throughout the host reticuloendothelial system. During a 10- to 14-day incubation period they multiply, culminating in the clinical onset of typhoid fever (93).

The possibility of alterations in motility of the gut as a contribution to virulence was recently investigated by Sninsky et al. (162). They demonstrated increases in repetitive bursts of action potentials in segments of isolated rabbit ileum when exposed to the cell-free supernatant of a culture of *C. jejuni* cells. This disturbance of myoelectic activity has been demonstrated with other enteric pathogens, most commonly invasive pathogens (27). Such disturbances are thought to enhance the virulence of these organisms through disordered peristalsis and a resulting increased resistance to flow, which favor microbial proliferation.

Attempts have been made to understand the invasive properties of *C. jejuni*. *C. jejuni* and *C. coli* invade the intestinal epithelium of infant mice (126), but most work has been with in vitro systems. Manninen et al. (100) demonstrated that *C. jejuni* organisms associate with and penetrate HeLa cells. Newell et al. (125) showed that clinical isolates invaded HeLa cells more readily than did organisms isolated from water. As yet, the properties of the organisms that confer invasion have not been determined, and correlations have not been made between clinical presentation and the ability to invade HeLa cells.

ANIMAL MODELS OF *CAMPYLOBACTER* ENTERITIS

Clear definition of the pathogenic mechanisms of *C. jejuni* has been hampered by the absence of a simple animal model. Initially, the standard animal models used with other enteric pathogens were found to be negative for this organism. *C. jejuni* has been shown to be negative in the Sereny test for invasiveness (100), and most investigators report no fluid accumulation in ligated rabbit ileal loops inoculated with viable organisms. Therefore, workers in the field had to identify new animal models that could be used to study this pathogen. As recently reviewed by Newell (122), a variety of animal species and experimental procedures have been evaluated in an attempt to establish a system to study *Campylobacter* spp. Because most of these models are too cumbersome (e.g., calves) or expensive (e.g., rhesus monkeys), they are impractical for use in most laboratories. However, several of these models are notable in that they mimic *Campylobacter* enteritis in humans. Prescott fed gnotobiotic beagle puppies with *C. jejuni*, and they subsequently developed mild diarrhea. Dogs sacrificed 43 h after inoculation had colitis consisting of neutrophil infiltration of the lamina propria, exfoliation of the surface epithelium, and loss of goblet cells with hypertrophy of the glands (141). Fitzgeorge et al. (50) used young rhesus monkeys, which also developed disease very similar to that seen in humans. Although the symptoms were mild, the animals had associated bacteremia and prolonged intermittent excretion of the organism in the feces. After recovery, animals challenged with the same strain of *C. jejuni* remained asymptomatic, had no associated bacteremia, and excreted the organism for only 3 days.

Numerous attempts have been made to develop a simple animal model of disease with broad application. Ruiz-Palacios et al. (147), Sanyal et al. (152), and Welkos (186) used 3-day-old or newly hatched chicks, which developed illness. These models promised to be more useful than large-animal models, but results have varied among laboratories (100).

Caldwell et al. (32) used the removable intestinal tie adult rabbit diarrhea (RITARD) procedure (164, 165) to produce disease in 1-kg rabbits. After a surgical procedure that results in a temporary small-bowel obstruction, the rabbits develop mucous diarrhea, with or without bacteremia, and abnormal intestinal histology very similar to that described in humans. The RITARD procedure is useful for studies of pathogenic mechanisms and immune responses, but is not suitable for screening large numbers of strains for differences in virulence factors.

Presently no established small-mammal model exists that mimics human disease in the absence of previous treatment or surgical procedure. Simple intragastric inoculation of adult mice (49) and hamsters (66) results in transient colonization without symptoms. This colonization can be prolonged in adult mice by pretreatment with antibiotics, which alters the normal colonic flora (49). Blaser et al. (14) showed that oral infection of adult mice does not induce disease, but is associated with colonization and bacteremia. Caldwell et al. (31) treated 1-kg rabbits with cimetidine to block gastric acid secretion, and intragastrically injected up to 10^9 CFU with bicarbonate. These rabbits shed *C. jejuni* in their feces for up to 3 weeks without symptoms. The same strain and dose of *C. jejuni* caused mucous diarrhea when the challenge was performed by the RITARD procedure (32). Similar findings have been reported in laparotomized hamsters that received *C. jejuni* inoculations directly into the intestine (66).

Kazmi et al. (77) have developed an infant-mouse model, which is presently the simplest of the in vivo systems. Of 42 infant mice challenged intragastrically with one of three strains of *C. jejuni*, 36 developed severe diarrhea. Minor differences were seen among the strains, but they were not statistically significant. These results followed virulence enhancement of the organisms by the addition of 1% iron dextran to the inoculum prepared by serial intraperitoneal passage in adult mice. It should be noted, however, that Stewart-Tull et al. (168) also decreased the 50% lethal dose of *C. jejuni* by intraperitoneal passage in infant mice. These results were ascribed to the effects of endotoxin in the inoculum because the organisms did not replicate in vivo.

TOXINS OF *CAMPYLOBACTER*

One important mechanism by which bacterial enteropathogens induce diarrhea is through the production of potent toxins. Bacterial toxins in general have been conveniently classified as either membrane damaging, such as hemolysins and phospholipases, or intracellular acting, such as the toxins produced by *Corynebacterium diphtheriae*, *V. cholerae*, and *Shigella dysenteriae* (115). The latter group is probably directly associated with the mechanisms for inducing diarrhea. These toxins are proenzymes that share several modes of action. They bind to specific receptors on the plasma membrane (GM1 ganglioside for cholera toxin [CT]), enter the cell and, once inside, interact with intracellular targets such as intracellular adenylate cyclase (CT and the

heat-labile toxin [LT] of *E. coli*) or the 60S subunit ribosome (shiga toxin). With CT this interaction causes fluid secretion, but shiga toxin causes the shutdown of protein synthesis and cell lysis (115).

C. jejuni is now known to produce at least two exotoxins: a heat-labile cytotonic or enterotoxin (CJT) and a cytotoxin (71). As described above, *Campylobacter* enteritis can present as a variety of disease syndromes, ranging from watery diarrhea to a dysentery characterized by bloody mucus and leukocytes in the stool. It is possible that one or both of these toxins could be responsible for these symptoms. Ruiz-Palacios et al. (151) found a difference in the frequency of isolation of enterotoxigenic *C. jejuni* organisms between patients with diarrhea and asymptomatic carriers. More recently, Klipstein et al. (84) was able to correlate enterotoxin and cytotoxin production with different manifestations of disease. However, it is premature at present to define the role of these two toxins in human disease.

Enterotoxin

There is recent evidence that strains of *Campylobacter* isolated from patients with watery diarrhea produce an enterotoxin that is in many ways similar to CT. This similarity includes induction of secretory diarrhea by stimulating adenylate cyclase activity in the intestinal mucosa and disrupting the normal ion transport in the enterocytes (151). Although CJT has not yet been fully characterized, it is known to be a large protein with a molecular weight in the range of 60,000 to 70,000 (107); it is heat labile and can be completely inactivated at 56°C for 1 h or at 96°C for 10 min. The toxin is partially inactivated at pH 4 and is completely destroyed at pH 2 and 8; toxic activity of the crude toxin is progressively lost after storage for 1 month at 4°C or for 1 week at −20 or −70°C (81, 82, 151). Concentrated enterotoxin preparations induce accumulation of fluid and electrolytes in both rat (48, 151) and rabbit (106) ileal loops but not in the infant mouse assay (151) and increase permeability in the rabbit skin test (106). The toxin causes cytotonic changes in confluent monolayer cell lines, inducing elongation of CHO cells and rounding of Y-1 mouse adrenal cells (59, 106, 151). There is consistent evidence that the intrinsic mechanism of action of the enterotoxin is mediated by an increase in the intracellular concentrations of cyclic adenosine monophosphate secondary to stimulation of adenylate cyclase (151).

Since CJT shares functional and immunological properties with CT and LT, the same assays have been applied to detect it. Tissue culture assays with either CHO (151) or Y-1 mouse adrenal cells (59, 106, 151) are convenient systems to test for toxin production. Double-sandwich enzyme-linked immunosorbent assay with GM1 ganglioside as the solid phase and anti-LT or anti-CT antibody as the second antibody to LT have been used to detect *C. jejuni* enterotoxin in supernatants of 24-h cultures (81, 82, 84, 106, 107).

The enzymatic activity of CJT is remarkably like that of CT and LT, and in fact this reaction can be inhibited by the addition of anti-CT antibody, thus stressing the functional and immunological relatedness with CT and CJT (83, 149, 150). One may speculate that it contains a subunit with enzymatic activity on the adenylate cyclase system, probably by adenosine diphosphate-ribosylating the guanosine triphosphate regulatory protein as for CT (115). CJT also possesses a B subunit which binds to specific receptors of the cell plasma membrane, thus permitting the internalization of the adenylate cyclase activator (83). This subunit binds specifically to the GM1 ganglioside in the same way that LT and CT-B subunits do, as was demonstrated with a GM1-enzyme-linked immunosorbent assay (83, 106).

There is no consensus regarding the conditions which maximize the yield of toxin production in *C. jejuni* cultures. Growth in a vitamin-supplemented medium followed by polymyxin treatment of the cells leads to a higher concentration of toxin in the culture filtrate (82). Polymyxin treatment presumably promotes release of the toxin from the periplasm of the cell. Other researchers have found that a medium rich in asparagine and serine enhances toxin production by the organisms (57). McCardell and Madden (105) found that CJT production is stimulated by the presence in the media of iron at a concentration beyond the level which stimulates growth of the organism, in contrast to Shiga toxin production, which has been shown to be optimal under iron-depleted conditions (180). Strains previously negative for toxin were shown to produce toxin when grown in media with increased concentrations of iron. Furthermore, cultures reverted to toxin negativity when grown in deferrated medium (105). Thus, evidence is accumulating that available iron may be important for virulence expression.

Workers in several laboratories have demonstrated immunological relatedness between CJT and both CT and LT. Preincubation of CJT with antisera to either CT or LT inhibits its cytotonic activity in CHO (151) and Y-1 (106) cells and its secretory effects in rat ileal loops (151). Partially purified preparations of *C. jejuni* holotoxin show lines of partial identity with CT (106) and LT (82, 83) by gel immunodiffusion. The B subunit of CJT also shows lines of partial identity with the B subunit of CT and LT (83). However, enzyme-linked immunosorbent assay with sera against either LT or its B subunit showed that it appeared to be more closely related immunologically to the LT β subunit than to the CT B subunit (82, 83).

Cytotoxin

There are data suggesting that *C. jejuni* and *C. coli* elaborate a cytotoxin which is toxic for a number of mammalian cells, including bovine kidney (L. C. Blankenship, S. E. Crawen, and S. R. Hopkins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, P3, p. 205), Vero (71), CHO (62, 71; Blankenship et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1982), and HeLa cells (62, 191). Toxin release is increased by treating the cells with polymyxin before centrifugation and filtration (62). This toxin is heat labile (100°C for 30 min) but stable at 60°C for 30 min (62, 191), trypsin sensitive (62, 191), and is not neutralized by immune sera to Shiga toxin (62), or the toxin of *Clostridium difficile* (62, 71). No correlation has been observed between cytotoxin production and biotype or serotype (71). The role of this toxin as a virulence characteristic in diarrheal disease remains unknown.

SURFACE STRUCTURES OF *CAMPYLOBACTER*

Studies of the structure of the *Campylobacter* cell at the molecular level have for the most part focused on the structure of the cell surface. This is because, for pathogenic bacteria, it is this surface that interacts directly with the human host. Indeed, it is the structure of the bacterial surface that allows the successful pathogen to avoid or to overcome the host defense mechanisms (160). Moreover, components of the bacterial cell surface and products such as toxins that cross this surface produce damage in the host. Therefore, to rationally devise procedures for immunoprophylaxis against pathogenic bacteria such as *Campylo-*

bacter spp., it is important to characterize these surface structures with regard to their role in pathogenesis and immunogenicity. Furthermore, for pathogens for which serological procedures are used for identification and epidemiological purposes, it makes sense to understand the molecular basis of surface structures for the particular serotyping scheme used. For the thermophilic campylobacters, the definition of the surface antigenic structure is of special interest, because these enteropathogens appear to be antigenically diverse. They have at least 50-heat stable serotypes in the scheme based on LPS (134) and more than 36 heat-labile serotypes in the Lior serotyping system based on flagellar antigens (94).

C. fetus Proteins and LPS

Owing to the importance of surface structures, considerable work has been accomplished during the past 15 years to determine the molecular nature of the LPS and protein components of the outer membrane of *Campylobacter* spp. Early studies on the surface structure of campylobacters concentrated on *C. fetus*. This organism was shown to produce a high-M_r protein antigen, which was first described by Myers in 1971 (118). Elegant studies by Winter and co-workers (110, 189) showed that this protein antigen took the form of a surface layer, which they described as a "micro capsule." The layer could be extracted from the cell surface by using glycine buffer at pH 2.2 and was shown to be composed of a protein of approximate subunit M_r 98,000. Layers of this type are more commonly referred to as S-layers, or regular surface protein arrays, and take the form of crystalline lattices that cover the entire cell surface (159). This demonstration of an S-layer on the surface of *C. fetus* was one of the first times that such a layer had been observed on a pathogen. For *C. fetus*, the layer was demonstrated to be antiphagocytic and may play an important role in the pathogenesis of *C. fetus* infections (108). Surface protein arrays have now been found on a variety of human and animal pathogens, and in at least one other case they appear to be essential for virulence (68).

Other studies showed that the flagella and the LPS of *C. fetus* were also important surface antigens (109, 188). Recent studies have shown that *C. fetus* LPS differs in structure somewhat from LPS of members of the family *Enterobacteriaceae*. Electrophoretic analysis of *C. fetus* LPS has shown that, in contrast to the smooth LPS of members of the family *Enterobacteriaceae*, which contains O polysaccharides of heterogeneous chain length (56), the O polysaccharides of *C. fetus* appear to be of quite homogeneous chain length (95, 97, 137). This structural characteristic may be related to the ability of this species to assemble an S-layer (7).

C. jejuni Surface Proteins

The surface structure of *C. jejuni* and its thermophilic relatives appears to differ markedly from that of *C. fetus* (178). Attempts to identify an S-layer on the surface of *C. jejuni* have been uniformly unsuccessful. However, extraction with glycine buffer (pH 2.2) does yield a surface protein of apparent subunit M_r 31,000 which appears to be common to the surface of many strains of *C. jejuni* that belong to a variety of heat-labile serotypes (96). It is obviously a candidate for inclusion in a *Campylobacter* vaccine. The structural form taken by this common antigen is not yet known, but the protein has recently been isolated with the aid of high-performance-liquid chromatography, and monoclonal antibodies to the 31,000-molecular-weight protein have been generated (L. A. Bath and T. J. Trust, manuscript in preparation). Detailed structural and immunochemical information on this potentially important surface antigen should be available shortly.

Another surface antigen is the major OMP. This protein, of M_r ca. 45,000, is likely to be the porin molecule, since it is transmembrane and peptidoglycan associated (95). Immunoblotting techniques have revealed considerable antigenic relatedness between the major OMPs of campylobacters belonging to diverse serotypes (16). However, although this molecule is also a candidate for inclusion in a vaccine, no information on the antigenic relatedness of surface-exposed domains of the molecule is yet available. This protein is likely to be important to the metabolism and growth of the *Campylobacter* cell by virtue of its putative porin function, but no evidence exists for any role in pathogenesis.

Other surface proteins contribute to the antigenicity of the *Campylobacter* cell. Indeed, immunofluorescence studies indicate that surface proteins can carry serospecific epitopes in cells in which the flagella are not involved. As yet, no specific surface or OMP has been shown to be solely responsible for serotypic specificity, although in one strain a 95,000 M_r OMP has been shown to contribute to its serospecific identity (96).

LPS

Electrophoretic and chemical analyses indicate that *C. jejuni* LPS is predominantly of the low-M_r lipoligosaccharide type (97, 119) found in such pathogens as *Neisseria*, *Haemophilus*, and *Bordetella* spp. (42, 67, 136). In contrast to these bacteria, in which the low-M_r LPS confers relatively few serotypes, the lipoligosaccharide of *Campylobacter* spp. appears to be antigenically diverse, conferring a large number of serotypes. This diversity is of considerable interest at the molecular level, because the heat-stable antigenic diversity of *Campylobacter* spp. must, for the most part, involve a difference in the quite small number of sugars found in the lipoligosaccharide. This is in contrast to an organism such as a *Salmonella* sp., in which the serological diversity results primarily from alterations in the sugar content and conformation of long O polysaccharide chains. The molecular elucidation of this antigenic diversity awaits detailed chemical analyses. The role of this lipoligosaccharide in *Campylobacter* pathogenesis also awaits clarification, but the lipid A portion of the molecule appears similar to that of other gram-negative bacteria (103).

Flagellar Protein

The motility of *Campylobacter* spp. is due to one or more flagella consisting of a filament 20 nm in diameter, a hook and basal body, and a large disk associated with the end of the hook region and covering the basal body (117). The flagellum, by virtue of its proteinaceous nature and its exposed surface location, is also an important surface antigen of *C. jejuni* (95). In a gastrointestinal pathogen such as *Campylobacter* spp., the colonization of mucus is likely to be an important step in pathogenesis. As stated above, motility is likely to play a major role in this mucus colonization, so that flagella or flagellin epitopes must be considered potential targets against which to direct host antibodies in immunoprophylactic measures. Certainly, convalescent human serum contains antibodies to flagellin (116, 123, 124), and some of these antibodies are directed against surface-located flagella epitopes (S. M. Logan and T. J. Trust, in preparation). It is likely, however, that these surface-

exposed epitopes will be mostly strain specific. In hyperimmune rabbit antiserum, flagellin is also a major surface protein antigen, and again antibodies appear to recognize surface-located epitopes (96). Depending on the flagellum preparation used in the immunization procedure, the antibodies produced are cross-reactive with flagella of other serotypes. In other cases, the antibodies formed are specific to flagella of the homologous strain. This accounts for the observation that in some (187) but not all of the heat-labile serogroups (L. A. Bath and T. J. Trust, unpublished observation), the flagella appear to carry the serospecific epitopes. In one case, the serospecific epitope has been shown to be dependent on conformation (L. A. Bath, S. M. Logan, and T. J. Trust, manuscript in preparation).

The flagella of *C. jejuni* are unsheathed and, depending on the strain studied, have a subunit protein M_r of 57,000 to 66,000. Analysis by CNBr fragmentation has shown that the *C. jejuni* protein differs from the *C. fetus* protein, but that considerable structural similarities occur among the *C. jejuni* flagellins (S. M. Logan and T. J. Trust, submitted for publication). Immunochemical analysis has revealed the presence of primary-sequence, non-surface-exposed epitopes shared by *C. jejuni* flagellins and *C. fetus* flagellins. There are also primary-sequence, non-surface-exposed epitopes that show limited strain cross-reactivity and still others that appear to be strain specific (Logan and Trust, submitted). Most recently, the production of flagella has been shown to be subject to phase variation by the demonstration of Fla^+-to-Fla^- switching (30) and by the ability of some strains of *C. jejuni* to produce flagella of different antigenic specificities (Bath et al., in preparation). If this is shown to be true, the antigenic picture of the *C. jejuni* cell will become even more complex.

IMMUNE RESPONSE TO *CAMPYLOBACTER* INFECTION

Several epidemiological studies have been used to suggest that immunity against *C. jejuni* in humans is acquired as a consequence of one or more infections. For example, Glass et al. (55) were able to demonstrate a progressive decrease in the illness/infection ratio with increasing age among children in Bangladesh. Black et al. (8) recently reported similar results among children in Peru. The development of immunity was also suggested by Blaser et al. (10), who conducted an age-stratified analysis of antibodies to *C. jejuni* and found elevated levels in healthy children from Bangladesh compared with those in the United States. Blaser et al. also showed that persons who drink raw milk regularly and presumably have multiple exposures to *C. jejuni* have persistent elevations in anti-*Campylobacter* immunoglobulin G (IgG) levels and little or no illness compared with persons who drink raw milk for the first time. These data also suggest that repeated exposure may lead to the acquisition of immunity (13). Furthermore, several reports have documented prolonged excretion or symptoms, or both, in immunodeficient patients. These are most commonly patients with IgA deficiency which suggests a primary role for humoral immunity against *Campylobacter* infection (73, 113).

The evidence for the acquisition of immunity as a result of natural infections in children has been substantiated in laboratory studies among volunteers in the United States. Black et al. (8) performed an experiment in which adult volunteers ingested 10^8 to 10^9 *Campylobacter* organisms in milk. Seven previously ill subjects from this group were rechallenged with 10^9 homologous organisms, along with 12 nonexposed individuals. In this experiment, none of the previously challenged volunteers became ill, but 6 of the 12 control volunteers developed diarrhea.

Rechallenge experiments with *C. jejuni* have also been done in animal models. Caldwell et al. (31) found that colonization of rabbits by gastric inoculation (164) with 5×10^8 live organisms did not cause disease, but protected the rabbits upon rechallenge by the RITARD procedures. Similar protection was obtained by Ruiz-Palacios et al. (148), who showed that animals that had been challenged by the RITARD process were protected during a second RITARD challenge. In both of these rabbit rechallenge experiments, blood cultures were negative. Colonization time dropped from 2 to 3 weeks in nonimmune animals to 1 to 2 days in immune rabbits. Shortened intestinal colonization time was also demonstrated in rechallenged rhesus monkeys (50).

The mechanism responsible for the rapid elimination of organisms or asymptomatic infection is presently unknown. This review previously showed that antibodies in serum are formed against flagella and other surface antigens that may be involved in colonization. Antibodies against LT were also detected in serum samples from patients infected with enterotoxigenic *Campylobacter* spp. (149). Studies by immunoblotting techniques have yet to be applied to evaluation of mucosal secretion samples obtained after *Campylobacter* infections, but Ruiz-Palacios et al. (148) used the ELISA to show significant increases in specific secretory-IgA in the intestines of challenged rabbits. ELISA techniques have also been used to characterize human serum antibody responses to campylobacterial enteritis. Blaser et al. (13) used the enzyme-linked immunosorbent assay to evaluate serum samples obtained from patients during the course of their illness, from healthy controls, from persons exposed in an outbreak but who remained well, and from persons who regularly drank raw milk. Raw-milk drinkers had elevated levels of *C. jejuni*-specific IgG antibodies, but normal levels of IgM and IgA. Patients with diarrhea developed rising antibody titers (IgA, IgM, and IgG) in their serum during week 2, compared with controls, whose titers remained constant. The IgG and IgM titers remained elevated at 45 days postinfection, but approached normal levels by 90 days. The IgA titers, by comparison, fell to normal levels more rapidly. A similar pattern was observed in persons exposed but not ill, although their titers were lower. Kaldor et al. (75) obtained similar results from patients after acute infection, but noted a poor antibody response in patients with prolonged or relapsing diarrhea.

More recently, Mascart-Lemone et al. (101) used solid-phase radioimmunoassays to study IgA antibody to *C. jejuni* in serum samples from almost 400 healthy people and approximately 60 patients presenting with acute enteritis. In healthy individuals, the frequency of detection of IgA antibodies to *Campylobacter* spp. in serum increased significantly with age, from less than 10% in children under 1 year to 37% in 20-year-old adults and 75% in elderly people. In these healthy people, anti-*Campylobacter* IgA was mainly monomeric IgA1. However, in patients with polymeric IgA1, the form synthesized primarily in the intestinal lamina propria, was predominant. The response in patients was short lived, but as yet no one has studied the duration of protective immunity to *Campylobacter* spp. and associated it with antibody response.

GENETIC CONTROL OF VIRULENCE FACTORS

Genetic analysis of the many determinants that contribute to the virulence of a microorganism is a powerful method for better understanding its pathogenesis. Unfortunately, unlike

studies of the genetics of other enteropathogens such as *E. coli* and *Shigella* and *Salmonella* spp., studies of the genetics of *C. jejuni* are in their infancy.

The chromosome of *C. jejuni* has been physically characterized to have an average moles percent G+C content of 30.1 to 33.0 and to be 80 to 85% the size of the chromosome of *E. coli* B (6, 130). However, unlike the situation for other enteric pathogens, no method has been described for the exchange of chromosomal DNA between strains of *C. jejuni*. Techniques of DNA transformation have not been developed, and although lysogenic phages have been reported (25, 33, 34, 144), no method of generalized transduction exists. Conjugative plasmids have been described, but mobilization of the *C. jejuni* chromosome by these plasmids in a fashion analogous to Hfr formation in *E. coli* has not been demonstrated. Furthermore, these plasmids are transferable only to *Campylobacter* spp. and not to *E. coli*. Conversely, no plasmids from *E. coli*, including those of the so-called wide-host-range compatibility groups, have been successfully transferred into *Campylobacter* spp. As a result of these technical deficiencies, no information is available on the organization of the *C. jejuni* chromosome.

Plasmids

Plasmids have been shown to specify virulence determinants in a variety of bacteria (for a review, see reference 47). Among these traits are adherence to epithelial cells, invasiveness, toxin production, iron sequestration, resistance to the bactericidal effect of serum, and resistance to antimicrobial compounds. Although many workers have described the physical isolation of plasmids from *C. jejuni* (2, 5, 24), only antibiotic resistance and, in certain special cases, enterotoxin production have been shown to be plasmid encoded.

Both Taylor et al. (172, 173) and Tenover et al. (174) have independently described conjugative plasmids of approximately 57 kilobases encoding tetracycline resistance. In a later study, Tenover et al. (176) isolated tetracycline resistance plasmids ranging in size from 2.0 to 162 kilobases. Despite the differences in molecular mass and restriction enzyme patterns, these plasmids showed a surprisingly high degree of nucleotide sequence similarity with one another. The gene-encoding tetracycline resistance has been cloned from one of these plasmids (171) and was found to lack homology to the previously described tetracycline resistance genes of *E. coli* (Diane Taylor, personal communication). This suggests a different origin for the evolution of resistance genes in *C. jejuni*.

Recently, Lambert et al. isolated a strain of *C. coli* harboring a 47.2-kilobase conjugative plasmid encoding both kanamycin and tetracycline resistance (T. Lambert, G. Gerbaud, P. Trieu-Cuot, and P. Courvalin, Ann. Inst. Pasteur, in press). The mechanism of kanamycin resistance has been characterized as a 3'-aminoglycoside phosphotransferase of type III, an enzyme never before identified in gram-negative bacteria. These workers have cloned and sequenced this kanamycin resistance determinant from *C. coli* and compared its nucleotide sequence with those of similar kanamycin resistance genes cloned from *Streptococcus* and *Staphylococcus* spp. (Lambert et al., in press). Except for small insertions and a few codon changes, all three genes showed almost complete sequence homology. These data suggest that *C. coli* may have acquired this kanamycin resistance gene from the gram-positive bacteria. In light of these data, it is interesting to note that Kotarski et al. (85), working with an independently isolated kanamycin-tetracycline resistance plasmid, have preliminary data suggesting that their kanamycin resistance determinant may be capable of translocation in *C. jejuni*. The identification of a transposon in *Campylobacter* spp. would have an enormous impact on the development of techniques to study the genetics of this organism.

As described above, several researchers have identified a choleralike enterotoxin produced by strains of *C. jejuni* (81, 106, 107, 151). Since similar enterotoxins have been shown to be plasmid encoded in *E. coli* (63, 161), Lee et al. (92) surveyed 30 enterotoxigenic isolates of *C. jejuni* for plasmids and found that only 61% harbored demonstrable plasmid DNA. Therefore, the presence of plasmids does not always correlate with enterotoxin production, and toxin production is presumably encoded on the chromosome in many, if not most, strains. However, in two clinical isolates from distinct geographical areas, enterotoxin production could be conjugally transferred along with tetracycline resistance to nontoxigenic strains of *C. fetus*. *C. fetus* transconjugants were capable of transferring these plasmids (pGK103 and pGK104) to a nontoxigenic strain of *C. jejuni*, rendering the recipient strains tetracycline resistant and enterotoxigenic. These results suggest that these plasmids encode the structural genes for toxin production, or that they have regulatory sequences capable of turning on toxin production in both *C. jejuni* and *C. fetus*. No nucleic acid homology has been demonstrated between pGK103 or the chromosome of nonplasmid-containing enterotoxigenic *C. jejuni* strains and gene probes for the A and B subunits of CT (J. Kaper and E. Lee, unpublished data) or the genes encoding the LT of *E. coli* (128; E. Lee, unpublished data). The genes specifying enterotoxin production may have evolved from a source other than the functionally homologous genes in *V. cholerae* and *E. coli*. Interestingly, there appears to be no nucleic acid homology between the enterotoxin of *S. typhi* and CT, despite the immunological similarities among these enterotoxins (Stanley Falkow, personal communication).

Tenover et al. (174) reported that a tetracycline resistance plasmid, pFKT1025, shows homology to *C. jejuni* DNA from tetracycline-sensitive, plasmidless isolates. Similarly, Lee et al. (92) showed that the enterotoxin-tetracycline resistance plasmid pGK103 shows homology to DNA isolated from an enterotoxigenic, tetracycline-sensitive, plasmidless strain of *C. jejuni*. It is tempting to speculate that there may be a general class of conjugative plasmids in *C. jejuni* that are capable of recombining with a region(s) of the chromosome and mobilizing these chromosomal sequences into other strains in a fashion analogous to F or R prime plasmids of *E. coli*. If this proves to be true, such plasmids could be useful in the development of a system of genetic exchange within *Campylobacter* spp.

Chromosomal Control of Virulence

Although enterotoxin is probably encoded on the chromosome in most cases, the only other potential virulence determinant studied with relation to genetic control is the flagellum. Several workers have compared the virulence of wild-type, motile *C. jejuni* with that of spontaneously isolated nonmotile, aflagellated mutants. Newell et al. (123) fed mice mixtures of strains 81116 Fla^+ and Fla^-, and found that only Fla^+ cells could be recovered in the feces of the animals. Similarly, when human volunteers were fed a mixture of strain A3249 Fla^+ and Fla^-, all *C. jejuni* isolated from subsequent stool cultures were motile (Robert Black,

personal communication). When Caldwell et al. (30) infected rabbits by the RITARD procedure with the Fla^- mutants of both of these strains, they could isolate only motile *C. jejuni* 96 h after infection, suggesting that the motility phenotype is unstable. These workers demonstrated a bidirectional switch in the expression of flagella in both of these strains. The frequency of the switch, as determined with motility medium, was 5.9×10^{-3} in the Fla^+-to-Fla^- direction and 8.0×10^{-7} in the Fla^--to-Fla^+ direction. Although they found that the Fla^+ form clearly predominated in vivo in the rabbit, their data could not distinguish a change in the switching frequency in vivo from a positive selection for the few flagellated organisms in the population. Although no molecular data are currently available on the nature of this variation, it is tempting to draw analogies to similar regulatory switches in other microorganisms.

Switches are becoming increasingly recognized as a mechanism by which pathogenic bacteria modulate certain surface properties. The classic example is that of phase variation in *Salmonella typhimurium* (90, 169). *S. typhimurium* can alternate its flagellar antigens between two distinct types, possibly in response to the host immune system. The elegant studies of Simon and co-workers (155–157, 193) determined that the regulation occurs by physical rearrangements of the coding DNA. Another more recently discovered example is that of the pili of uropathogenic *E. coli* (46), which are thought to be important early in infection as adhesins to epithelial cells. Later in infection, when the organism has entered the bloodstream, the expression of pili is turned off, presumably because the piliated organism is more susceptible to phagocytosis. Other examples can be found in *Neisseria gonorrheae* (26, 114, 167), *Bordetella pertussis* (135, 185), and *Borrelia hermsii* (112). The role of variable expression of flagella in the pathogenesis of *C. jejuni* remains to be elucidated.

It is somewhat surprising that only one report describes the cloning of *C. jejuni* chromosomal genes in *E. coli*. In a study designed to ascertain the feasibility of expressing *C. jejuni* genes in *E. coli*, Lee et al. (E. C. Lee, R. I. Walker, and P. Guerry, Can. J. Microbiol., in press) cloned the genes for γ-glutamyl kinase and γ-glutamyl phosphate reductase by complementation of the corresponding defects in *E. coli* (*proB* and *proA*). Although the data are not conclusive, expression of these genes in *E. coli* seems to occur by a *C. jejuni* promoter. However, the same workers were unable to identify clones capable of complementing a leucine auxotrophic marker in *E. coli*, suggesting that not all *C. jejuni* genes may express in *E. coli*. Thus, problems of cross-species expression of genes may complicate the analysis of virulence determinants by recombinant DNA techniques.

PERSPECTIVE

The related vibrios described by King (79, 80) have shown themselves to be a group of several distinct microorganisms with characteristics both similar and unique among enteric pathogens. The significant impact of diarrhea lends urgency to efforts to determine the epidemiology, physiology, and pathophysiology of these organisms.

Some of the mysteries of *Campylobacter* enteritis are beginning to give way to understanding. It now seems possible that *C. jejuni* may have an assortment of virulence options for toxigenic or invasive diarrhea as well as asymptomatic infection. The genetic and environmental effects on the interplay of enterotoxin, cytotoxin, and presently unknown invasive factors of a particular strain must be determined and then related to immune and other host factors that affect the outcome of disease.

The initial process by which *C. jejuni* colonizes the intestine before appearance of disease symptoms induced by toxins or other mechanisms may involve flagella, LPS, or other surface structures. Since protective immunity against *Campylobacter* spp. is associated with rapid clearance of the organism from the intestine, it seems that this immunity is directed against colonization antigens. Elucidation of the role of each of these antigens in pathogenicity and the way each affects intestinal immunity remain for future study. Indeed, the antigenic complexity of the *Campylobacter* cell presents workers with a number of interesting challenges. Elucidation of the importance of these components in the pathogenesis of *Campylobacter* infections requires the development of molecular genetic systems and simple virulence assays. One thing that can be predicted is that *Campylobacter* spp. will continue to surprise.

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